



## Vegetatively propagating forest trees

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### Abstract

Propagation by seeds gives rise to individuals which are all genetically different from each other. By contrast, asexual or vegetative propagation consists in duplicating, theoretically unlimitedly, genotypes while preserving through mitotic divisions their original genetic make-up, and consequently all their individual characteristics. This is essential to ensure the transfer of economically important traits which are under non-additive control. Vegetative propagation can be applied to any individual that does not produce fertile seeds, either because it has not entered the mature reproductive stage yet, or due to unfavorable environmental conditions. Its usefulness is obvious for research as well as for operational activities, depending on the ultimate objectives and on the most suitable strategies to meet the goals. Conventional nursery techniques and *in vitro* culture can be used for vegetatively propagating forest tree species. The respective pros and cons of these various vegetative propagation methods, which can synergistically complement each other, are considered, mainly from an operational viewpoint. Species characteristics and cost effectiveness must be taken into account for applications while pondering the real advantages and limitations of vegetative versus seed-based propagation strategies in the general context of forest tree plantations.

**Keywords:** axillary budding, clone, cuttings, grafting, *in vitro* culture, organogenesis, self-rooted plants, somatic embryogenesis.

### Foreword

The 4th conference of the IUFRO working unit 2.09.02 has recently given us the opportunity to discuss the last advances of somatic embryogenesis (SE) and other vegetative propagation (VP) technologies applied to forest tree species. SE was given preponderant consideration during the past conferences of the IUFRO working unit 2.09.02 consistently with its affiliation to IUFRO unit 2.09.00 dealing with “Tree seed, physiology and biotechnology”. There is, however, a need to broaden the scope to other VP methods, formerly regrouped within the IUFRO working unit 2.01.17 “Physiology of vegetative reproduction” before its fusion with 2.09.2 in 2010. This will give the opportunity to widen the range of forest tree species amenable to different VP techniques, with special mention for those planted in tropical countries due to their worldwide importance. Organizing the venue of the conference in La Plata, which was attended by a great number of participants from the Northern but also from the Southern hemispheres, supports this view. Consistently, it seems relevant to reconsider the particularities and usefulness of VP as a whole, as well as the various ways it can be applied for managing and using sustainably forest tree resources in view of the forthcoming challenges of the 21st century. Improving, the sooner the better, the productivity and the quality of wood production in plantations, in order to lessen the pressure on the irreplaceable natural forest ecosystems that are more and more endangered, is a major issue. The purpose of this paper is to examine, from realistic and practical viewpoints, what can be expected from VP for meeting this goal with the shortest delays, emphasizing concrete outcomes rather than longer-term and more uncertain expectations.



## **Vegetative propagation: main features and current importance for forest tree plantations**

Seed-derived individuals are all genetically different from one another. By contrast, asexual or vegetative propagation (VP) consists in duplicating, theoretically unlimitedly, genotypes while preserving, through mitotic divisions, their original genetic make-up, and consequently all their individual characteristics. For several tree species, VP is a natural propagation procedure, be it by suckers (*Populus spp*, *Robinia pseudoacacia*), layers (*Thuja spp*, *Argania spinosa*) or even apomixis (*Citrus spp*, *Cupressus dupreziana*). Usually, the separation from the mother plant takes place once the offspring can rely on functional roots. VP can also be induced artificially for more specific purposes as described in this paper. It can be applied to any individual that does not produce fertile seeds, either because it has not yet entered the mature stage or, as is mostly the case for exotic species, due to unfavorable environmental conditions. By producing genetically identical offspring, VP is a way to improve wood population uniformity in yield and quality especially for plantations established in monoclonal blocks (Zobel and Talbert 1984, Ahuja and Libby 1993a, Lindgren 2002). Conversely VP is associated with a substantial impoverishment in genetic diversity, depending on the number and the genetic relatedness of the clones deployed. Contrary to propagation by seeds, VP cannot create any new genotypes. It must therefore be considered as a dead-end from a genetic improvement viewpoint, except when the clones produced are used for breeding through suitably established clonal seed orchards (CSOs) or for genetic engineering (White et al. 2007, Ahuja 2011, Harfouche et al. 2011).

## **Possible usefulness of VP for forest tree species**

VP can be used in forestry for different purposes (Libby 1974, Zobel and Talbert 1984, Ahuja and Libby 1993a) such as:

- *ex-situ* conservation of genepools which are endangered *in situ*, like in the case of *Cupressus dupreziana* which is restricted to very few individuals in its native Thassili mountains in Algeria. Thirty two genotypes were successfully grafted, physiologically rejuvenated and clonally propagated by rooted cuttings at the French R and D institute AFOCEL (Franclet 1977, Monteuuis, unpublished results). The rooted clones were then planted in different clonal test locations in France, which can be converted into CSOs for producing new genotypes and enriching thereby the narrow genetic diversity of this species in peril (Fauconnier 2012).
- Genetic enrichment or improvement through seeds produced from clones selected and then planted for this purpose within wisely designed CSOs.
- Assessing the influence of the environment (E) on field behavior of the clone which is liable to vary according to genotype (G), certain clones being more adaptable or plastic than others, as reflected by G X E interactions.
- Evaluation of various genetic parameters such as broad sense heritabilities, genetic correlations... (Burdon and Shelbourne 1974).
- Physiological studies, including phase change, resistance or tolerance to environmental constraints like frost, drought...
- Genetic engineering using somatic embryogenesis (SE), preferably when derived from single DNA-transformed cells, for regenerating with the higher chances of genetic and phenotypic stability completely transgenic trees (Trontin et al. 2007, Ahuja 2011, Hazubska-Przybył and Bojarczuk 2016). Nonetheless, it should be kept in mind that the few transgenes available to date are restricted to traits under monogenic control whereas most of the economically important forest tree features are assumed to be polygenically determined (Ahuja 2011, Harfouche et al. 2011). The risks of modifying host plant characteristics by genetic transformation should not be ignored. Society's reluctance and strict jurisdictions towards transgenic plants, trees included, are also increasingly interfering with genetic engineering activities.



- From an operational standpoint, the mass production of superior and uniform planting material in order to generate with the shortest delay a high yield of outstanding quality wood.

## VP techniques

Trees can be vegetatively propagated in bulk or as separate clones (Talbert et al. 1993, Ritchie 1994, Monteuuis 2016), by grafting or on their own roots, self-rooted, in nursery or *in vitro* culture, distinguishing between axillary budding, organogenesis and SE origins (Ahuja and Libby 1993a, Hartmann et al. 1997).

### Grafting

Grafting is a helpful alternative for cloning individuals that are reluctant to *de novo* organogenesis, i.e., adventitious rooting, budding and somatic embryogenesis. It consists in combining a root system, the rootstock, to an aerial part, the scion, to give rise to a bi-genotypic new individual. Grafted plants differ in this respect from self-rooted vegetative offspring, usually produced by rooted cuttings under suitable nursery conditions profitably equipped with a mist or fog-system (Hartmann et al. 1997). These are not required for grafting. Seedlings or clones can be utilized as rootstocks. The term homograft is employed when rootstock and scion are from the same species and heterograft when the two symbionts are from different species. Pros and cons of grafting have already been extensively described (Hartmann et al. 1997). It is used for instance in fruit tree orchards for increasing tree density and hence overall productivity by grafting on specific rootstock clones. The vigorous tap roots produced by seed-issued rootstocks can overcome the limitations of clones that are difficult to root or produce a deficient adventitious root system that is non-adapted to the planting site conditions. Operationally, grafting has had a tremendous impact on the industrial development of rubber tree clonal plantations which are all grafted (Masson and Monteuuis 2017). It is also widely used on a smaller scale for reproducing special ornamental growth habits, like witches' broom, 'compactum', 'nana' or 'pendulum' cultivars that could have resulted from an auto-maintained disease or from maturation-associated physiological deficiencies. Thus the weeping variety "pendulum" of *Sequoiadendron giganteum* could be maintained by heterografting onto *Sequoia sempervirens*, which was less vigorous than homografting (Monteuuis 1985), but disappeared after cloning by microcuttings (Franclet 1981). Grafting is also a means of producing clonal plants that do not delay the onset of flowering and seed or fruit production in seed orchards, as physiologically mature material can be propagated by grafting but not self-rooted (Borchert 1976, Hackett 1985, Bonga 1987). Its main usefulness in forestry is for breeding or for operational production of genetically improved seeds from judiciously set up CSOs, as well as for establishing *ex-situ* genepool conservation stands (Zobel and Talbert 1984, Ahuja and Libby 1993a, White et al. 2007). Grafting has also been used under certain circumstances for physiologically rejuvenating mature selected genotypes as a prerequisite to the true-to-type production of self-rooted clonal offspring (Franclet 1977, Cauvin and Marien 1979, Monteuuis 1985). The possibilities to graft miniaturized scions onto juvenile rootstocks *in vitro*, but also the most suitable "physiological window" for collecting the scion from the donor plant, have been assumed to play a key role in this regard (Monteuuis 2012). However, due to its particularities and constraints (Hartmann et al. 1997, Zobel and Talbert 1984), grafting is not compatible with large scale clonal forestry which consists of self-rooted clones exclusively.

### Self-rooted shoots produced by axillary budding

Cuttings, minicuttings and microcuttings differ from one another basically in the length of the shoots used to be rooted. They all result from the elongation of preexisting shoot meristems. Propagation by axillary



budding is hence the most natural and reliable artificial VP method, whose efficiency has already been proven in nursery as well as in *in vitro* conditions.

### Macropropagation

Propagation by rooted cuttings or minicuttings in nursery conditions, also referred to as macropropagation (Rauter 1983), is the most widely used technique for vegetatively mass producing forest trees. It has been proven to be more effective and efficient than tissue culture for a wider range of genotypes of several species, especially *E. urophylla* X *E. grandis* (Saya et al. 2008) and *Hevea brasiliensis* (Masson and Monteuuis in these proceedings). The main and first requisite is to make sure that the shoot (ramet) removed from the donor tree *in situ* (ortet) or stock plant remains alive long enough for proper rooting. Adventitious root formation is determined by endogenous and exogenous factors notwithstanding their likely interactions (Rauter 1983, Davis et al. 1988). Certain cells, assumedly in shoot perivascular tissues, must have the capacity to dedifferentiate and give rise to *de novo* produced root tips which are different in many respects, anatomically in particular, from the shoot structures from which they are derived (Davis and Haissig 1994). The basic requirements for mass propagating the genotypes selected by rooted cuttings are *i*) adapted nursery facilities, resorting to a reliable automatic mist or fog system for maintaining the cuttings alive during the length of time needed for roots to develop and to become functional and *ii*) have the proper know-how for inducing and sustaining a sufficiently high capacity for true-to-type cloning by rooted cuttings of the plant material (Rauter 1983, Hackett 1985, Hartmann et al. 1997). Starting in the late 70's, AFOCEL has had a pioneering influence in the development of efficient techniques for mass clonally propagating by rooted cuttings in nursery conditions mature ortets of various tropical and temperate tree species (Franclet 1977, 1981). The importance of shortening the distance within the donor plant between the shoots to be used as cuttings, and the roots, especially the tips from where the hypothetical rejuvenating hormones the cytokinins originate (George 1993), accounted for intensive and repeated shoot hedging and pruning practices (Franclet 1977, Hackett 1985, Monteuuis 1989). These methods were combined with serial rooting for regenerating new root systems with root tips closer to the shoots (Monteuuis 1993). This gave rise to the minicutting system: the  $n^{\text{th}}$  plant generation is serially produced from short axillary bottom shoots collected from the previous  $n-1^{\text{th}}$  generation of plants raised in small volume Melfert plugs before field planting (Monteuuis et al. 1987). Another advantage of this system is to save nursery or greenhouse space. Initially developed by AFOCEL for *Eucalyptus gunnii*, *E. globulus* and related interspecific eucalypt hybrids (Cauvin 1982, Chaperon et al. 1984) as well as for other temperate species like *Sequoia sempervirens* (Monteuuis et al. 1987), the minicutting propagation system has subsequently been applied with great success in warmer countries for (sub) tropical tree species. These encompass eucalypt clones and interspecific hybrids (Wendling and Xavier 2003, Titon et al. 2006, Saya et al. 2008), teak (Ugalde Arias 2013), *Gmelina arborea* and others of more local interest (Monteuuis 1993). Propagation by minicuttings is nowadays more and more used for reducing the cost of plants produced by advanced *in vitro* methods, especially SE (Thompson 2014, Bonga 2015, Georget et al. 2017). The benefits of advanced and sophisticated nursery equipment like aero and hydroponics seem however questionable, the priority being to produce at lower cost the quantity of good quality clonal offspring required. Moreover, macropropagation methods, no matter how elaborate, will always face limitations that micropropagation techniques can overcome (Monteuuis 2016).

### Micropropagation

Its main interest lies in the possibility to use *in vitro* culture for initiating the mass production by axillary budding from miniaturized ramets, the microcuttings, which are too tiny to survive in natural conditions. A microcutting or microshoot is a shoot portion that has at least one apical or axillary meristem. Its initial size





usually ranges between 1 and 2 cm but can be as small as 0.1mm when it is restricted to the shoot apical meristem (SAM). The pros and cons as well as the successive steps of micropropagation by axillary budding have been developed recently, emphasizing the benefits of using miniaturized ramets for mass clonally propagating true-to-type selected genotypes with greater efficiency than by more conventional methods (Bonga 1987, Monteuuis 1989, 2016). The microshoots can be maintained in the absence of any root and serially subcultured *in vitro* on proper culture media long enough to ensure their mass multiplication and their physiological rejuvenation needed for efficient adventitious rooting, preferably carried out in nursery conditions (Bonga 1982, Durzan 1984, Hackett 1985). As an illustration, a SAM-issued rejuvenated *Sequoiadendron giganteum* line and an *Acacia mangium* mature selected genotype introduced *in vitro* in 1986 and 1995 respectively have been maintained through serial subcultures up to now (Monteuuis et al. 2008, Monteuuis and Bon 2000). This prolonged subculture procedure had no noticeable effect on the growth and organogenic capacities but could be associated to a significant increase of DNA methylation compared to the same material grown outdoors (Monteuuis et al. 2008, 2009). The use of simple *in vitro* protocols and the possibility to mass micropropagate in a restricted space, year around, regardless of the local outdoor conditions can also reduce production costs (Monteuuis 2000). A comparative study made for teak within the same company in Sabah, East Malaysia showed that for more than 100 000 clonal offspring produced annually, micropropagation was more cost effective than nursery techniques (Monteuuis 2000). This was mainly due to the savings made on intensive and time consuming management of stock plants which are not needed in tissue culture. Lastly, being contamination-free, tissue-culture remains to date the only way to dispatch vegetative plant material for research as well as for operational and commercial purposes to any international destination. This is of determining importance for the rapid diffusion of the YSG BIOTECH TG1-8 teak clones from East Malaysia to various tropical countries worldwide (Goh and Monteuuis 2012, 2016, Monteuuis and Goh 2017).

### ***Self-rooted shoots produced by in vitro organogenesis***

Unlike propagation by axillary budding, organogenesis or *de novo* propagation requires first that particular cells from superficial tissues are able to dedifferentiate and then to reinitiate a new organogenic program leading to the formation of adventitious meristems that can subsequently develop into shoots (Durzan 1984, Thorpe and Patel 1986, Bonga and von Aderkas 1992). Because ontogenetic ageing is localized in the SAMs (Fortanier and Jonkers 1976), organogenesis-issued shoots can thus be assumed to be completely ontogenetically rejuvenated. *De novo* shoot formation can occur naturally, for instance from sphaeroblasts in certain tree species like beech or eucalypt. It can also be induced artificially in tissue culture from various organs which are usually associated with the first stages of the ontogeny like cotyledons, hypocotyls, epicotyls or primary needles for *Pinus spp.* (Dunstan and Thorpe 1986, Thorpe et al. 1991). Therefore, except for a few cases of morphological rejuvenation (Fouret et al. 1989, Bonga and Pond 1991, Dumas and Monteuuis 1991), very juvenile genotypes, too young to be reliably selected on their real genetic value, are mostly used for micropropagation by organogenesis. These primary explants are inoculated on suitable *in vitro* culture media enriched with high concentrations of growth regulators for inducing, directly or indirectly from a possible transitory callus, the formation of adventitious meristems, meristemoids or meristematic nodules (Dunstan and Thorpe 1986, Bonga and von Aderkas 1992). Only a part of these newly formed meristems will eventually elongate into shoots that can then be further micropropagated by axillary budding to be ultimately rooted and acclimated. Initially viewed as a highly efficient micropropagation technique (Thorpe and Biondi 1984, Dunstan and Thorpe 1986, Thorpe et al. 1991), it has become obvious that the expectations were far greater than the actual outcomes (Thompson 2014, Bonga 2015). This can be due to several reasons: *i*) only very juvenile genotypes can be *de novo* micropropagated, with noticeable variations of responsiveness between and within species at the family or individual levels (Durzan 1984, Thorpe and Biondi 1984, Hargreaves et al. 2005); *ii*) adventitiously produced juvenile clones can hardly be maintained in micropropagation the time needed for reliable field testing and cryopreservation of this

material has limitations (Bonga 2015); *iii*) the field behavior of the adventitiously-produced plants of different species was disappointing, showing growth, vigor and conformity abnormalities as well as symptoms of early physiological maturation (Bonga 1991, Gupta et al. 1991, Hargreaves et al. 2005), despite the organogenesis-induced ontogenetic rejuvenation; *iv*) the successive *in vitro* transfers and manipulations required are constraining and result in prohibitive production costs (Bonga 2015). For *Pinus radiata*, the species for which *de novo* micropropagation has been the more utilized so far, adventitiously-derived plants were reported to cost 7 times more than open-pollinated seedlings (Menzies and Aimers-Halliday 1997). This situation has warranted an increasing interest in the use of SE for mass clonal propagation (Hazubska-Przybył and Bojarczuk 2016).

### ***Somatic embryogenesis***

*In vitro* propagation by SE has already been largely documented (Thorpe 1995, Germania and Lambardi 2016, Hazubska-Przybył and Bojarczuk 2016). Briefly, it consists in producing embryos from somatic cells by mitotic divisions, hence preserving their original genetic make-up. This is the only and fundamental difference with zygotic embryos to which somatic embryos are identical in many other respects. SE is therefore a cloning technique. Except for rare occurrences of direct embryogenesis, including genotype-dependent cleavage polyembryogenesis (Durzan and Gupta 1987; Sharma and Thorpe 1995; Durzan 2008), the somatic embryos are formed indirectly usually after an intermediate callus stage artificially induced by the application of strong growth regulators which could cause somaclonal variations (Bairu et al. 2011). In the most favorable situations, undifferentiated cells of these calli can gradually evolve into somatic embryos characterized, similarly to zygotic embryos, by a shoot–root bipolar structure prefiguring the future plant (Thorpe 1995). This basically distinguishes somatic embryos from adventitious and axillary budding-derived microcuttings consisting of shoots from which adventitious roots must develop subsequently. When originating from a single cell, SE is the most striking and concrete illustration of cell totipotency (Durzan 1984, Thorpe 1995). SE remains the supreme and only way of achieving complete ontogenetic rejuvenation for the whole plant by virtue of its power to reset ontogenetic ageing to zero through the formation of embryonic structures that characterize the very first stages of ontogeny. The older and more developed the mother plant, the greater the magnitude of this ontogenetic rejuvenation. In this respect, *Hevea brasiliensis* (Carron and Enjalric 1985), *Quercus robur* (Toribio et al. 2004, San–José et al. 2010; Ballester and Vieitez 2012), *Quercus ilex* (Barra-Jiménez et al. 2014), *Eucalyptus globulus* and *E. saligna* × *E. maidenii* (Corredoira et al. 2015) deserve special consideration as SE could be obtained from sporophytic tissues of mature genotypes whereas in most cases, and especially in conifers, only certain immature or mature zygotic embryos respond positively (Thorpe 1995, Germania and Lambardi 2016, Hazubska-Przybył and Bojarczuk 2016). It can logically be assumed that ontogenetic and physiological rejuvenation are positively related (Borchert 1976, Fortanier and Jonkers 1976). This could explain that embling-derived *H. brasiliensis* trees showed a higher capacity for SE than their grafted same age, same size and same clone homologs (Lardet et al. 2009). The physiological rejuvenation associated with the SE-induced ontogenetical rejuvenation has also been proven helpful for further mass clonally propagating by rooted cuttings the few SE-derived industrial genotypes of *H. brasiliensis* that have been obtained (Masson et al. 2013). In *Abies nordmanniana*, SE has been adopted as the solution for overcoming the poor rooting rates and ageing-related plagiotropic effects associated with VP by rooted cuttings (Find 2016). Occasionally, emblings can also demonstrate a higher capacity for SE than usually expected for seedlings of the same age as observed for *Picea abies* (Ruaud et al. 1992, Harvengt et al. 2001) and *Picea glauca* (Klimaszewska et al. 2011). It has been hypothesized that this could be due to a possible SE-induced delay of physiological ageing (Klimaszewska et al. 2011, Bonga 2016). However, the fact that only particular embling-derived genotypes responded positively also suggest the influence of a genetic predisposition for SE.



For a long time considered to be the most promising and efficient cloning technology after the disillusion encountered with organogenesis (Hazubska-Przybył and Bojarczuk 2016), SE is still facing major scale-up hindrances. According to Thompson (2014), these are: *i*) a too limited effectiveness due to a strong genotype-dependent control of capacity for SE at the genus, species, provenances, family and individual levels; *ii*) lack of efficiency of the protocols used starting with the initiation and conversion rates, then affecting the quality of the emblings produced (Timmis 1998, Bonga 2016); *iii*) excessive production cost that cannot be offset by a high enough field superiority compared to much cheaper good quality seedlings. Emblings, at least in their first stages of development, have often been observed to grow slower than seedlings and to be prone to within sample variations according to species and procedures (Aronen 2016, Högberg 2016, Trontin et al. 2016a). Moreover, although completely ontogenetically rejuvenated by SE, emblings can exhibit several symptoms of early maturation such as premature flowering (Colas and Lamhamedi 2010, Breton, personal communication), lower ability for adventitious rooting (Högberg 2016)... This could be caused by non-optimal *in vitro* culture conditions liable to affect prematurely the sensitive and permeable cells from which SE and adventitious buds are derived (Meiland 1997, von Aderkas and Bonga 2000, Högberg et al. 2001). For instance, culture media are empirically made with a restricted list of components that are liable to interact and vary during the course of time, independent of the physiological requirements of the more or less organized cells or group of cells involved at different stages of the SE process. This will always be a constraint, even though SE efficiency can very likely be increased by improving the current protocols (Park and Bonga 2011), notwithstanding possible genotype x culture medium interactions. There is still a need to fine tune the first steps of the SE procedure for higher effectiveness, efficiency and cheaper costs in very realistic conditions with big enough and replicated representative samples before focusing on automation with the hope of reducing costs (Timmis 1998, Thompson 2014). Prohibitive production cost for SE-derived materials of insufficient quality remains the major deterrent to industrial use of SE for large scale plantation programs. Mass multiplying by rooted cuttings the insufficient numbers of expensive emblings managed as responsive stock plants has been viewed as an option for producing more planting material at cheaper cost (Lelu-Walter et al. 2013, Thompson 2014, Bonga 2015). The possibility to cryopreserve the SE-derived clones and a higher number of representatives per clone are the main advantages of such a procedure, compared to direct and more effective as less genotype-dependent bulk propagation by rooted cuttings of more genotypes from the same genetic background (Talbert et al. 1993, Ritchie 1994). Similarly to organogenesis, SE scaling-up may have been anticipated with excessive optimism based on findings drawn from limited scale research experiments too much disconnected from operational conditions (Thompson 2014). The fact that SE of *Picea abies* benefitted from thirty years of heavy research investments that did not result in any industrial application (Lelu et al. 2013) is enlightening in this respect. In addition to economic considerations and except for rare species, it should be emphasized that only very juvenile genotypes that are predisposed to SE and too young to be reliably selected on their field value, are liable to respond successfully to SE (Dunstan and Thorpe 1986, Bonga 2016, Hazubska-Przybył and Bojarczuk 2016). These aspects should be seriously considered when selecting material to be propagated by SE or other means.

### Material to be vegetatively propagated

The material to be vegetatively propagated must:

- i. thrive under planting site conditions, untested exotic origins being more prone to inadaptability to local environment than native ones. In this respect, it is symptomatic to note the preference given to clones of exotic species, with special mention for eucalypts, to start new industrial forest plantations in places where these materials have never been introduced before, without even considering the potential of native species to meet plantation objectives, at least partly. Planting local species



- contributes to the preservation of the natural biodiversity, bearing in mind that the genetic erosion associated with the use of clones compared to seedlings should not be minimized.
- ii. be well known and prized by the end-users. As an illustration, AFOCEL has during the late 70's-80's strongly and dynamically promoted clonal plantations of exotic species to be intensively managed in order to feed the increasing needs of pulp and paper mills (Afocel 1982). *Sequoia sempervirens* for instance was chosen for its specific properties and more particularly its high growth rate, low resin content and long fibers that are valuable assets for papermaking. Efficient and innovative propagation techniques were developed for mass producing by rooted cuttings in nursery conditions clones from mature selected Plus trees of 100 yr-old and older with average rooting rates of more than 80% (Monteuuis et al. 1987). Experimental plots were set up and 40 years of field observations have demonstrated the remarkable vigor of this species (Harvengt et al. 2013). In spite of such success at the experimental level, *Sequoia sempervirens* has never been and is still not planted at an industrial scale to be used by the pulp and paper industry, at least in Europe.
  - iii. generate the greatest genetic gain, the magnitude of which depends on the range of variability for traits of commercial interest within the seedling population. The wider this variability, the more room for selection at higher intensity by focusing on the few best genotypes which are far distant from the average for the most prized criteria. The stronger the selection pressure, the greater the expected returns (Zobel and Talbert 1984, Ahuja and Libby 1993a, White et al. 2007).
  - iv. have a sufficient capacity to be cost-efficiently mass clonally propagated true-to-type: the quality-price ratio of the planting material has a determining impact on return on investment for forest plantations in accordance with the market value of the end product.

### **High value vs pulpwood species**

High value timber species will definitely fetch higher market prices than pulpwood species at the end of a growing period or rotation, with the objective of producing with the shortest delay the highest volume of the best quality wood, in accordance with the relevant business plans. Promoting fast growth is also a concern of prime importance for high value timber because it reduces weeding and maintenance costs, it shortens the rotation and thus assures sooner delivery of valuable logs, the bigger the more prized. But volume is not the only criterion: log quality matters also and to a far greater extent than it does for pulp or fuelwood tree species. Clear bole length, shape, deformities like knots, buttresses, forks and branches have a big impact on log processing into sawn timber, veneer peeling and even splicing, by reducing waste and by enhancing the quality of the final product. For species like teak for instance, wood characteristics and aesthetic features, especially for refined furniture or yacht deck end-uses, have also a great market value influence (FAO 2009, Kollert and Cherubini 2012, Ugalde Arias 2013). The most highly valued products combine the most prized log and wood traits and it is very unlikely that these can be captured altogether by seed propagation. For such materials, and when possible, cloning seems therefore greatly justified, the superior cost of the clonal offspring compared to seedlings being offset by the much higher value of the final product. The situation is quite different for tree species planted mainly for pulp or fuelwood production. These are mainly propagated by seeds despite overly optimistic speculations that could have been drawn from the particularities of other species in different contexts. In *Abies nordmanniana* and *Picea abies*, true-to-type cloning by SE has been limited to the production of nicely shaped Christmas and ornamental tree clones respectively as these end-uses generate much higher added value than wood production (Lelu et al. 2013, Find 2016, Högborg and Varis 2016).





### *Mature vs juvenile genotypes*

The foregoing arguments plead strongly for the selection of candidate plus trees (CPT) for cloning (**Fig. 1**). A CPT is individually or mass selected based on phenotypic traits irrespective of its breeding value which characterizes an elite tree (Zobel and Talbert 1984). For reliable selection, a CPT requires to be developed hence old enough to express its superiority for as many traits of high commercial value as possible (Bonga 1982, Zobel and Talbert 1984, Ahuja and Libby 1993a). The rationale of preferring to clone mature selected genotypes rather than juvenile ones has already been largely argued (Zobel 1981, Bonga 1982, 1987), but is less and less acknowledged when not simply ignored (Hazubska-Przybył and Bojarczuk 2016), except for a few persevering research teams (Ballester et al. 2016, Klimaszewska and Rutledge 2016, Trontin et al. 2016b). Several traits of major economic importance like volume, branchiness, clear bole length and shape, wood characters especially for high value timber species are the priority criteria used for CPT selection. These criteria can be combined under the terminology of multi-trait selection for upgrading the superiority of the candidate clone (Zobel and Talbert 1984, White et al. 2007).

In teak more specifically, the initial multi-trait-based phenotypic selection of the CPTs is upgraded by taking into consideration wood value indications obtained by non-destructive core sampling as well as genotypic information drawn from DNA molecular markers (Goh et al. 2007, **Fig. 2**). Such multi-trait mass selection is immediate, very practical and efficient regardless of information on the additive vs non additive control, in other words on the heritability of the traits desired or on the genetic pedigree and relatedness of the CPTs, notwithstanding the importance of such indications for safe clonal deployment (Zobel 1981, Zobel and Talbert 1984, Ahuja and Libby 1993a). This clonal forestry strategy, starting from mature selected CPTs, is quite rational and attractive in many respects, at least theoretically (Bonga 1982, Libby and Rauter 1984, Ahuja and Libby 1993a). Practically, it can be implemented very quickly, providing suitable true-to-type and cost effective mass cloning methods are available, as demonstrated recently for teak (Goh et al. 2005, 2007, Goh and Monteuuis 2016, **Fig. 3**). This is the only but unavoidable limitation, the capacity for true-to-type cloning of most tree species by *de novo* organogenesis, *i.e.*, adventitious rooting, budding and somatic embryogenesis being severely antagonized by ageing (Bonga 1982, Hackett 1985, Bonga and von Aderkas 1992). The possibilities of physiologically rejuvenating mature selected genotypes for overcoming this cloning reluctance have been investigated during many years, but not enough, too superficially and without the discernment required (Franclet 1981, Monteuuis 1989, Bonga and von Aderkas 1993). For instance reinvigoration and rejuvenation have been for a long time confounded (Pierik 1990, Monteuuis et al. 2011a, Wendling et al. 2014). Likewise, investigations on physiological ageing should have given more consideration to likely interactions between variations in physiological state and growth activity, distinguishing between resting, bud break and active elongation periods in relation to phenology, seasonal variations and endogenous rhythms (Monteuuis 1989, Monteuuis et al. 1995, Mankessi et al. 2009). Practically, rejuvenation successes have been too anecdotal and limited in scope to warrant their application to the true-to-type mass clonal propagation of mature CPTs, especially for tree species of major economic importance (Bonga et al. 2010). These activities on physiological rejuvenation of mature selected genotypes, with the possibility to resorting to SE for complete rejuvenation, as a requisite for efficient mass clonal propagation by rooted cuttings have progressively been abandoned, although maybe too soon with reference to *Hevea brasiliensis* (Masson et al. 2013, Masson and Monteuuis in these proceedings).

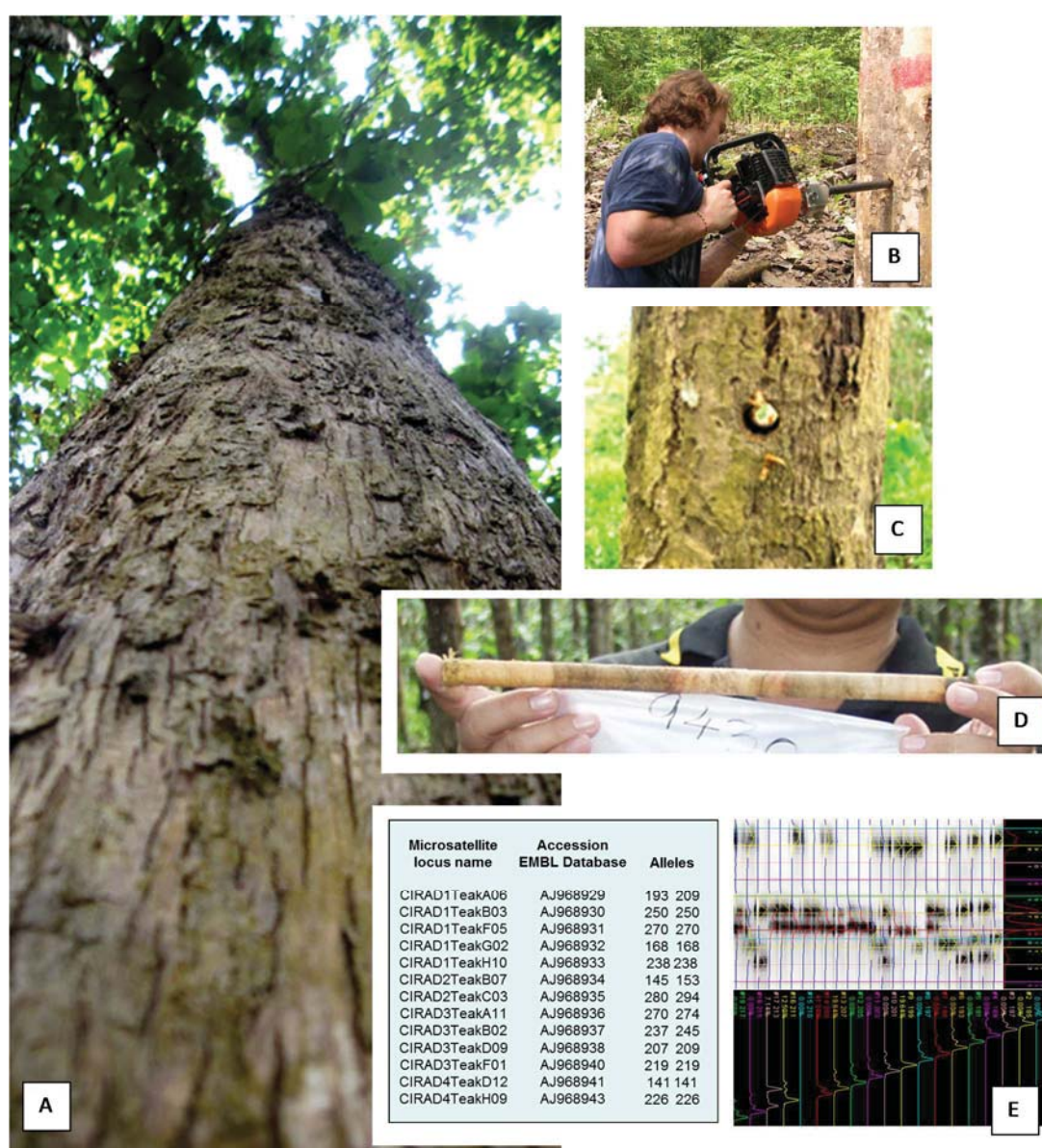
Another option is to start the clonal selection from a large population of clones initially derived from young selected genotypes and serially propagated by rooted cuttings to be established within properly designed clonal tests (Kleinschmit 1974, Menzies and Aimers-Halliday 1997). The inferior clones are rogued progressively as they express their field characteristics while developing in the course of time. The superior genotypes remaining can then be mass cloned, provided that the rooting ability of the cuttings can be maintained over the years (St. Clair et al. 1985, Mason et al. 2002). Attempted initially on a large scale with Norway spruce clones and later with other coniferous species, this strategy has proven to be too time, space



**Figure 1.** Candidate Plus Trees (CPTs) for cloning in even-aged family planted stands: *Eucalyptus spp.*: 6 months-old in Pointe-Noire, Congo (1a) and 2-year old in Budkinon, Mindanao, Philippines (1b); *Tectona grandis*, 22 year-old, Tchorogo, Togo (2); unexpected occurrence of a natural *Acacia auriculiformis* X *A. mangium* hybrid within a 2 year-old *A. mangium* family plot, Lai Uyên, Binh Duong, Vietnam (3); 10 year-old *Acacia mangium* in Luasong Forestry Center, Sabah, East Malaysia (4).



and money consuming to be sustained (Kleinschmit 1974, Kleinschmit and Schmidt 1977, Högberg and Varis 2016). Therefore, and because of an increasing interest in advanced biotechnology rather than for outdoor experimentation, research on forest tree cloning has been focused on the development of protocols for mass clonally propagating by SE very young *i.e.* mature or even immature genotypes (Thorpe et al. 1991, Bonga 2015, Masson and Monteuuis in these proceedings). The relevant prevailing argument is that efficient breeding programs, supported by molecular marker-assisted selection and cryopreservation, do not warrant any more work on true-to-type cloning of mature selected CPTs (Park and Gupta 2012, personal conversations during the 2<sup>nd</sup> IUFRO 2.09.02 conference in Brno, Park et al. 2016).



**Figure 2:** Multitrait mass selection of mature teak CPTs (A) upgraded by the use of non-destructive core sampling wood analysis procedures (B, C, D) and microsatellite molecular markers (E).





**Figure 3:** The mature selected teak genotypes are rejuvenated, then mass clonally propagated by *in vitro* micropropagation (A) or by macropropagation, with the possibility for the microshoots only to be dispatched worldwide under proper conditioning (B, C). The microcuttings are rooted and acclimatized (D) under similar nursery conditions as for macropropagation by rooted cuttings (E). After proper raising (F, G), the clonal offspring are ready to be field planted.

Although interesting, this view seems in some respects to be overly speculative and optimistic. First of all, each seed-issued genotype is unique by virtue of the DNA recombinations resulting from the crossing overs occurring during meiosis, over which breeders have no control. Therefore, the new genotypes derived from advanced breeding programs will be different from any other genotype and hopefully better than a particular outstanding CPT. However there will always be a risk that this will not happen and that the time, energy, land and cost invested may not pay off ultimately, especially for selections based on a combination of several traits which are not necessarily genetically linked and are assumed to be under non additive control (Zobel and Talbert 1984, Cornelius 1994, White et al. 2007). What can be realistically expected from marker assisted selection in such particular cases, as well as the relevant cost and time frame needed remain after 20 years of investment still questionable (Muranty et al. 2014). Another major concern is the strong additive genetic control of SE initiation capacity (Park et al. 1998, Klimaszewska et al. 2007), at least for certain species. This tends to promote the selection in the first place of SE responsive families, with the possibility to further select clones within these families resorting to cryopreservation technology (MacKay et al. 2006, Park and Bonga 2011, Bonga 2016). The preponderant importance given to SE initiation capacity, even for orienting breeding programs (Park et al. 1998, Klimaszewska et al. 2007) favors the selection of heritable characters at the expense of traits under non additive control, which could have a great economic impact (Zobel and Talbert 1984, Timmis 1985, Timmis et al. 1987). Growth and form for instance have been observed to vary substantially within progenies between genetically related individuals (Zobel and Talbert 1984, Chaix et al. 2011, Monteuuis et al. 2011b, **Fig. 1**). The comparison between container-grown white spruce seedlings and embling clones for various traits, at the between and within family levels, are quite enlightening, within family differences of mean values being greater among embling clones than the mean value among the seedlings (Lamhamedi et al. 2000). CPT-derived clones differ also from the average of the population, but primarily in their superiority in economically important traits. Initially selecting genotypes on their capacity for SE initiation may result in the elimination of clones with superior characteristics if these and SE responsiveness are not positively correlated. Furthermore, there is the risk of adverse selection in case of negative correlations (Haines and Woolaston 1991, Adams et al. 2016, Högborg and Varis 2016). In addition, there are still some uncertainties as regards possible risks of genetic instability associated with the cryopreservation of the SE-derived clones during the time required for reliable field testing (Park and Bonga 2011, Bonga 2016, Hazubska-Przybył and Bojarczuk 2016). Such problems do not exist for clonal forestry programs based on individual selection of mature CPT, as has long ago been demonstrated for a large number of planted tree species and more recently for teak (Ahuja and Libby 1993b, Lindgren 2002, Monteuuis and Goh 2017). In any case, priority for selecting CPT must be given to economically important traits over ease of clonal techniques that need as much as possible to be adapted to the particularities of the CPT rather than the reverse.

### Deployment of VP-issued wood populations

The rationale of propagating trees vegetatively has been to plant populations that can generate, with the shortest delays, the highest volume of premium and uniform wood quality to best meet end-user expectations. Several success stories have clearly demonstrated during the past decades the practical advantages for some forest tree species of preferring clones to seedlings for producing wood (Ahuja and Libby 1993b, Talbert et al. 1993). Mature selected CPTs were mass clonally propagated true-to-type by rooted cuttings to be field-planted. The number of clones as well as the size and the design of the monoclonal blocks planted at the same time must be adapted to the particularities of each clone and to between clone genetic relatedness. All this has been abundantly documented (Ahuja and Libby 1993a and b, Lindgren 2002). Production of self-rooted clones remains however strongly influenced by the genetic origin of the ortet and the negative effect of physiological ageing on adventitious rooting capacity. This has warranted, for certain species, the mass propagation by rooted cuttings of seedlings in their early stage of development. These, presumably being of high genetic value but available in insufficient numbers, are too



young to be individually selected on their field characteristics. They are, therefore, propagated in mixture, as a bulk without keeping the individual genotypic identity during the successive cycles of propagation (Talbert et al. 1993, Ritchie 1994). This will ultimately result in a drastic and uncontrolled reduction of the initial genetic diversity of the bulk population (Monteuuis 2016). Besides and as previously argued, there are always risks of propagating genotypes that are too young to be field tested due to uncertainty about correlations between economically important traits and VP capacity (Haines and Woolaston 1991). Therefore, bulk propagation differs basically from the clonal option, the latter offering the possibility of deploying clones in a mixture, as a polyclonal variety consisting of a well-known number of representatives of each clone. All these reasons could explain why polyclonal varieties, especially when derived from mature selected CPTs, have gradually supplanted bulk propagated VP populations. This strategy has so far mainly prevailed for teak clonal forestry, which is still in its infancy and hence facing time limitations for properly testing the clones, as it should be done, notwithstanding the time, space, manpower and money constraints associated with the process (Zobel and Talbert 1984, Lindgren 2002). The most widely used teak polyclonal variety consists of 8 mature selected clones (Goh and Monteuuis 2012, Monteuuis and Goh 2017, **Fig. 4**). These are mostly planted in a mixture with the purpose of buffering possible risks of clone inadaptability to the planting site conditions, as compared to monoclonal blocks which are more uniform, for better or worse.

Independent of the intensification of clonal forestry activities with broadleaf tree species mainly, the strong interest devoted to SE during the past decades for coniferous species of the northern hemisphere has logically given rise to the emergence of new deployment strategies with special emphasis on Multi Varietal Forestry (MVF) (Park 2002). Being SE-based, MVF faces the previously argued limitations, in particular a too low initiation and plant conversion rates, and risks of genetic instability in long-term cryopreserved embryogenic lines (Park 2002, Klimaszewska et al. 2007). Primarily driven by SE capacity, MVF may also miss traits of great economic impact that are under additive control but not positively correlated with the ability for SE initiation, in addition to all the valuable characters which are non-additively controlled and as such will be excluded. This constitutes a major drawback compared to the returns expected from clonal forestry programs based on the multi-trait mass selection of mature CPTs. Resorting to molecular markers for refining the selections (Park et al. 2016), mixing emblings and seedlings in plantations (Park 2002, Thompson 2014, Adams et al. 2016), and preferring to use the name varieties instead of clones (Klimaszewska et al. 2007, Park and Bonga 2011, Park et al. 2016), have been proposed for wider acceptance and promotion. This makes the field situation quite confusing and heterogeneous, especially as regards to the genetic composition of the relevant tree populations, which matters the most *in fine* (Burdon and Aimers-Halliday 2006). All this contrasts with the specificities of clonal forestry, whose field benefits, when wisely implemented, have already been clearly established (Zobel 1981, Ahuja and Libby 1993a,b).

### Concluding remarks

The decision to vegetatively propagate forest trees must be pondered due to the practical consequences in several domains. Species characteristics as well as the returns expected and the best ways to meet plantation objectives deserve major consideration. Attractiveness for the latest biotechnologies supported by too far reaching and overly optimistic speculation, should not be given excessive importance at the expense of more concrete and adapted options whose efficiency have already been demonstrated. Owing to its particularities and higher cost, VP seems to be more suitable for the true-to type mass clonal propagation of mature CPTs selected for their outstanding superiority in traits of great economic value, the more the better. Preference must be devoted to the more efficient but not necessarily the more advanced VP methods, or combinations thereof, for reaching this goal. A likely prerequisite for that is the physiological rejuvenation of the mature selected genotypes. Again, SE due to its capacity to achieve complete rejuvenation deserves special consideration. Within such a context and due to all these VP limitations, the



advantages associated with the propagation of forest tree species by seeds should be kept in mind. In addition to cost efficiency, propagation by seed is the more natural and powerful way of creating the genetic diversity so much needed for the environment and for tree genetic improvement.

**Acknowledgements:** the author is greatly indebted to Dr Jan Bonga for improving the final writing of this paper.



**Figure 4:** Four-year-old monoclonal blocks of YSG Biotech teak clones produced from mature selected CPT on steep slopes of southern Java, Indonesia. Maintenance was limited to weeding the first year, in absence of any pruning operation. The trees display the YSG Biotech TG1-8 characteristic features i.e. excellent straightness, reduced lateral branching and high leaf density accounting for increased photosynthesis and impressive growth rate.



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